

1           METHOD AND APPARATUS FOR SELECTIVE BIOLOGICAL MATERIAL

2                           DETECTION

3  
4  
5           Reference to Related Applications:

6           This application is a continuation-in-part of 09/550,779, filed  
7           on April 17, 2000, which is a continuation-in-part of S.N.  
8           09/218,827, filed on Dec. 22, 1998 and now U.S. Patent  
9           6,051,388, having an issue date of April 18, 2000, the contents  
10          of which are herein incorporated by reference.

11  
12           Field of the Invention

13           This invention relates to the detection of pathogenic  
14           microorganisms, or biological materials, and more particularly  
15           relates to a composite bioassay material useful for the  
16           detection of particular toxic substances, its method of  
17           manufacture and method of use, wherein the composite material  
18           is particularly useful for food packaging and the like, and is  
19           capable of simultaneously detecting and identifying a  
20           multiplicity of such biological materials.

21  
22           Background of the Invention

23           Although considerable effort and expense have been put  
24           forth in an effort to control food borne pathogenic

microorganisms, there nevertheless exist significant safety problems in the supply of packaged food. For example, numerous outbreaks of food poisoning brought about by foodstuffs contaminated with strains of the E-Coli, Campylobacter, Listeria, Cyclospora and Salmonella microorganisms have caused illness and even death, not to mention a tremendous loss of revenue for food producers. These and other microorganisms can inadvertently taint food, even when reasonably careful food handling procedures are followed. The possibility of accidental contamination, for example by temperature abuse, in and of itself, is enough to warrant incorporation of safe and effective biological material diagnosis and detection procedures. Further complicating the situation is the very real possibility that a terrorist organization might target either the food or water supply of a municipality or even a nation itself, by attempting to include a pathogenic microorganism or toxic contaminant capable of causing widespread illness or even death. If, by accident or design, the food supply of a particular population were to be contaminated, it is not only imperative that the population be alerted to the contamination, but it is further necessary that the particular contaminant be quickly and precisely pinpointed so that appropriate countermeasures may be taken.

Thus, if it were possible to readily substitute standard

packaging materials with a flexible material capable of

- 1) quickly and easily detecting the presence, and
- 2) indicating the particular identity of a variety of pathogenic biological materials, a long felt need would be satisfied.

#### Description of the Prior Art

The Berkeley Lab Research News of 12/10/96, in an article entitle "New Sensor Provides First Instant Test for Toxic E.Coli Organism" reports on the work of Stevens and Cheng to develop sensors capable of detecting E. Coli strain 0157:H7. A color change from blue to red instantaneously signals the presence of the virulent E. Coli 0157:H7 microorganism. Prior art required test sampling and a 24 hour culture period in order to determine the presence of the E. Coli microorganism, requiring the use of a variety of diagnostic tools including dyes and microscopes. An alternative technique, involving the use of polymerase chain reaction technology, multiplies the amount of DNA present in a sample until it reaches a detectable level. This test requires several hours before results can be obtained. The Berkeley sensor is inexpensive and may be placed on a variety of materials such as plastic, paper, or glass, e.g. within a bottle cap or container lid. Multiple copies of a single molecule are fabricated into a thin film which has a

1 two part composite structure. The surface binds the biological  
2 material while the backbone underlying the surface is the  
3 color-changing signaling system.

4 The Berkeley researchers do not teach the concept of  
5 incorporating any means for self-detection within food  
6 packaging, nor do they contemplate the inclusion of multiple  
7 means capable of both detecting and identifying the source of  
8 pathogenic contamination to a technically untrained end user,  
9 e.g. the food purchaser or consumer.

10 Wang et al, in an article entitled "An immune-capturing  
11 and concentrating procedure for Escherichia coli 0157:H7 and  
12 its detection by epifluorescence microscopy" published in Food  
13 Microbiology, 1998, Vol. 15 discloses the capture of E. coli on  
14 a polyvinylchloride sheet coated with polyclonal anti-E. coli  
15 0157:H7 antibody and stained with fluorescein-labeled anti-E.  
16 coli 0157:H7. After being scraped from the PVC surface, the  
17 cells were subjected to epifluorescence microscopy for  
18 determining presence and concentration. The reference fails to  
19 teach or suggest the concept of incorporating any means for  
20 self-detection within food packaging, nor does it contemplate  
21 the inclusion of multiple means capable of both detecting and  
22 identifying the source of pathogenic contamination to a  
23 technically untrained end user, e.g. the food purchaser or  
24 consumer, and especially fails to disclose such detection

1 without the use of specialized detection techniques and  
2 equipment.

3 U.S. Patent 5,776,672 discloses a single stranded nucleic  
4 acid probe having a base sequence complementary to the gene to  
5 be detected which is immobilized onto the surface of an optical  
6 fiber and then reacted with the gene sample denatured to a  
7 single stranded form. The nucleic acid probe, hybridized with  
8 the gene is detected by electrochemical or optical detection  
9 methodology. In contrast to the instantly disclosed invention,  
10 this reference does not suggest the immobilization of the probe  
11 onto a flexible polyvinylchloride or polyolefin film, nor does  
12 it suggest the utilization of gelcoats having varying  
13 porosities to act as a control or limiting agent with respect  
14 to the migration of antibodies or microbial material through  
15 the bioassay test material, or to serve as a medium for  
16 enhancement of the growth of the microbial material.

17 U.S. Patent 5,756,291 discloses a method of identifying  
18 oligomer sequences. The method generates aptamers which are  
19 capable of binding to serum factors and all surface molecules.  
20 Complexation of the target molecules with a mixture of  
21 nucleotides occurs under conditions wherein a complex is formed  
22 with the specific binding sequences but not with the other  
23 members of the oligonucleotide mixture. The reference fails to  
24 suggest the immobilization of the aptamers upon a flexible

polyvinylchloride or polyolefin base material, nor does it suggest the use of a protective gelcoat layer which acts as a means to selectively control the migration of antibodies and antigens, or to serve as a medium for enhancement of the growth of microbial material.

### Summary of the Invention

The present invention relates to packaging materials for food and other products, along with methods for their manufacture and use. The presence of undesirable biological materials in the packaged material is readily ascertained by the consumer, merchant, regulator, etc. under ordinary conditions and without the use of special equipment. A multiplicity of biological materials threaten our food supply. The present invention provides a unique composite material capable of detecting and identifying multiple biological materials within a single package. The biological material identification system is designed for incorporation into existing types of flexible packaging material such as polyvinylchloride and polyolefin films, and its introduction into the existing packaging infrastructure will require little or no change to present systems or procedures. Thus, the widespread inclusion of the biological material detecting system of the instant invention will be both efficient and

1 economical.

2 In one embodiment of the invention the biological material  
3 detecting system prints a pattern containing several antibodies  
4 or aptamers, derived from plant or animal origins, onto a  
5 packaging material which is usually a type of polymeric film,  
6 preferably a polyvinylchloride or polyolefin film and most  
7 preferably a polyethylene film which has undergone a surface  
8 treatment, e.g. corona discharge to enhance the film's ability  
9 to immobilize the antibodies upon its surface. The agents are  
10 protected by a special abrasion resistant gel coat in which the  
11 porosity is tailored to control the ability of certain  
12 antibodies, toxic substances, etc. to migrate therethrough.  
13 Each antibody is specific to a particular biological material  
14 and is printed having a distinctive icon shape. The detection  
15 system may contain any number of antibodies capable of  
16 detecting a variety of common toxic food microbes; although any  
17 number of microbes may be identified via the inventive concept  
18 taught herein, for the purpose of this description, the  
19 microbes of interest will be limited to E.Coli, Salmonella,  
20 Listeria and Cyclospora.

21 An important feature of the biological material detection  
22 system is its all-encompassing presence around and upon the  
23 product being packaged. Since the biological material  
24 detecting system is designed as an integral part of 100% of the

1 packaging material and covers all surfaces as utilized, there  
2 is no part of the packaged product which can be exposed to  
3 undetected microbes. In the past, the use of single location  
4 or *in situ* detectors have left a majority of the area around  
5 and upon the packaged product exposed to undetected microbes.  
6 This greatly increased the chance that a spoiled or tainted  
7 product might be inadvertently consumed before the toxic agent  
8 had spread to the location of the *in situ* detector. The  
9 biological material detection system of the present invention  
10 avoids this problem by providing a plurality of individual  
11 detectors per unit area which are effective to insure positive  
12 detection of any pathogenic microorganisms within the product  
13 being tested. In order to be effective a particular degree of  
14 sensitivity is required, e.g. the detecting system must be  
15 capable of positively identifying one microbial cell in a 25  
16 gram meat sample. In a preferred embodiment, four detectors  
17 per square inch of packaging material surface have been  
18 utilized, and in a most preferred embodiment nine or more  
19 detectors per square inch are incorporated upon the film's  
20 surface.

21 By use of the biological material detection system of the  
22 present invention a packager or processor can independently  
23 determine the multiplicity and identity of those biological  
24 materials against which the packaged product is to be



1 protected. Although it is envisioned that the large majority  
2 of biological material detection treated packaging will be  
3 generic to approximately four of the most common microbes, the  
4 system will nevertheless allow each user to customize the  
5 protection offered to the public.

6 The biological material detecting system will not merely  
7 detect the presence of biological materials, it will also  
8 identify the particular biological materials located in a  
9 packaged product. This unique feature allows for the immediate  
10 identification of each particular biological material present  
11 since the antibodies are specific to a detector having a  
12 definitive icon shape or other identifying characteristic.  
13 Although the end use consumer is primarily interested in  
14 whether a food product is, or is not, contaminated per se, the  
15 ability to detect and identify the particular biological  
16 material immediately is of immeasurable value to merchants,  
17 processors, regulators and health officials. The ability to  
18 immediately identify a toxic material will lead to greatly  
19 reduced response times to health threats that might be caused  
20 by the biological material and will also enhance the ability  
21 for authorities to locate the source of the problem. The  
22 biological material detecting system of the present invention  
23 exhibits an active shelf life in excess of 1 year under normal  
24 operating conditions. This enhances the use of a biological

1 material detection system on products which are intended to be  
2 stored for long periods of time. If these products are stored  
3 so as to be ready for immediate use in some time of emergency,  
4 then it is extremely beneficial to definitely be able to  
5 determine the safety of the product at the time that it is to  
6 be used.

7 One particularly important feature of the biological  
8 material detecting system of the instant invention is its  
9 ability to quantitatively sensitize the reagents so as to  
10 visually identify only those biological materials which have  
11 reached a predetermined concentration or threshold level which  
12 is deemed to be harmful to humans.

13 For example, almost all poultry meat contain traces of the  
14 salmonella bacteria. In most cases, the salmonella levels have  
15 not reached a harmful level of concentration. The biological  
16 material detecting reagents are designed to visually report  
17 only those instances where the level of concentration of  
18 biological materials are deemed harmful by health regulatory  
19 bodies.

20 The method of production of the biological material  
21 detecting system is designed to be easily incorporated within  
22 the packaging infrastructure of existing systems without  
23 disruption of the systems or the procedures under which they  
24 are operating. The biological material detecting system can be

1 incorporated onto packaging films which are produced by the  
2 packager, or those which are supplied by a film manufacturer.  
3 The apparatus necessary for applying the biological material  
4 detecting system may be easily located at the beginning of any  
5 continuous process such as printing or laminating and will  
6 operate as an integral part of an existing system.

7 The biological material detecting system of the instant  
8 invention represents an entirely new packaging material which  
9 is designed to inform the consumer of the presence of certain  
10 biological materials or pathogens present in food stuffs or  
11 other materials packaged within the detecting system. The  
12 system is designed so that the presence of a biological  
13 material is presented to the consumer in a distinct,  
14 unmistakable manner which is easily visible to the naked eye.

15 Recent outbreaks of E.Coli and other health hazards have  
16 presented serious problems to the general population and have  
17 raised concerns regarding the safety of the food supply.

18 It is an objective of the present invention to provide a  
19 biological material detecting system for protecting the  
20 consumer by detecting and unmistakably presenting to the  
21 untrained eye visual icons on the packaging material which  
22 signify the presence of a number of pathogens in the food stuff  
23 or other materials which are at a level harmful to humans.

24 It is another objective of the instant invention to

1 provide a bioassay material wherein an antigen detecting  
2 antibody system is immobilized upon the surface of a flexible  
3 polyolefin film.

4 It is still another objective of the instant invention to  
5 provide a bioassay material wherein an antigen detecting  
6 antibody system is immobilized upon the surface of a flexible  
7 polyvinylchloride film.

8 It is a further objective of the invention to provide a  
9 biological material detecting system which is so similar in  
10 appearance and utilization that its use, in lieu of traditional  
11 packaging materials, is not apparent to the food processor or  
12 other packagers.

13 A still further objective of the present invention is to  
14 provide a biological material detecting system which is cost  
15 effective when compared to traditional packaging materials.

16 It is still another objective of the present invention to  
17 provide a biological assay material for protecting the consumer  
18 by detecting and unmistakably presenting to the untrained eye  
19 one or more visual icons on a packaging material which signify  
20 the presence or absence of a particular toxic substance.

21 Other objectives and advantages of this invention will  
22 become apparent from the following description taken in  
23 conjunction with the accompanying drawings wherein are set  
24 forth, by way of illustration and example, certain embodiments

of this invention. The drawings constitute a part of this specification and include exemplary embodiments of the present invention and illustrate various objects and features thereof.

#### **Brief Description of the Figures**

Figure 1 is a cross-sectional interpretation of an antibody sandwich immunoassay device;

Figure 2 is a cross-sectional interpretation of a single ligand assay;

Figure 2A is a cross-sectional interpretation of a single ligand assay including a chromogenic ligand;

Figure 3 is a diagrammatic representation showing the functioning of a single ligand assay;

Figure 4 is a cross-sectional interpretation of an antibody sandwich immunoassay including a scavenger system for microbial quantification;

Figures 5 and 6 are a diagrammatic representation showing the functioning of a sandwich assay/scavenger system;

Figure 7 is a planar view of an example of icon placement and printing;

Figure 7A is an example of a typical code of identification applied to the icon pattern;

Figure 8 is the result derived from EXAMPLE 2 and exemplifies capture sensitivity of a single ligand treated

polyvinylchloride film;

Figure 9 is a block diagram of the apparatus illustrating the process steps for forming a sandwich assay;

Figure 10 is a block diagram of the apparatus illustrating the process steps for forming a single ligand assay;

Figure 11 is a diagrammatic representation showing first and second layers printed onto a base layer film;

Figure 12 is a diagrammatic representation showing a first layer printed onto a base layer film;

Figure 13A illustrates a biological material assay in an icon shape visible on a film;

Figure 13B illustrates a loss of visibility of an icon shape on a film;

Figure 14A illustrates a biological material assay in a first icon shape surrounded by a second icon shape on a film;

Figure 14B illustrates a loss of visibility of a first icon shape on a film;

Figure 15A demonstrates an antigen presented to a homogeneous conjugate blend;

Figure 15B demonstrates an antigen displacing a peptide/dye conjugate;

Figure 15C demonstrates an antigen binding to an immobilized ligand.

## Description of the Preferred Embodiment(s)

Referring now to Figure 1, the detection and identification of various biological materials in packaged foods or other products is accomplished by the use of antibodies which are specific to the biological material being sought. Specific antibodies, defined as capture antibodies, are biologically active ligands characterized by their ability to recognize an epitope of the particular toxic substance being tested. These capture antibodies are selected from such materials as antibodies, aptamers, single stranded nucleic acid probes, lipids, natural receptors, lectins, carbohydrates and proteins. In one embodiment of the invention, the capture antibodies are arranged with unique icon shapes and in particular patterns. The capture antibodies are immobilized to the polymer film. An agarose gel coat containing detector antibodies is printed in register above the capture antibodies. A protective gel coat completes the construction of the packaging material. The gel coat constituting the inner layer, e.g. that layer which is next to the packaged product, is a special type of gel coat or an equivalent thereto which has sufficient porosity to allow toxic molecules, known as antigens, to migrate through it to an antibody "sandwich" laminated between the polymer film and the gel coat. The special gel coat has sufficient abrasion resistance to prevent

1 exposure of the reagents to the product. The special gel coat  
2 useful in the invention is a readily available coating commonly  
3 utilized in the food industry to coat candies and the like,  
4 e.g. coated chocolates to prevent them from melting on one's  
5 hands. Migration of antigens is driven by capillary action and  
6 normally reaches a state of equilibrium within a 72 hour time  
7 period. In a particularly preferred embodiment, when operating  
8 within a temperature range of 4 - 25 degrees Celsius, an  
9 initial positive reading can be obtained within 30 minutes, and  
10 the test continues to yield results for about 72 hours. Upon  
11 migrating through the special gel coat the antigen enters an  
12 agarose gel film which has surfactant properties, contains free  
13 detector antibodies, and also contains one or more ingredients  
14 designed to enhance the growth of microbial materials, e.g.  
15 nutrients such as sorbitol, NOVOBIOCIN, CEFIXIME and TELLURITE  
16 which increase the growth rate and ease isolation of E. Coli  
17 0157H. If the antigen encounters a species of antibody which  
18 is specific to an epitope thereof, it will then bind to it  
19 forming a detector/antibody complex. Once bound thereto, the  
20 bound antigen/antibody complex becomes too large to migrate  
21 back through the special gel coat due to its inherent fine  
22 porous structure. This insures that pathogenic material can  
23 not migrate back into the product being tested. Continuing  
24 pressure toward equilibrium from capillarity will tend to move



1 the antigen, with its bound antibody, through a second gel coat  
2 layer and into an area of the flexible polyvinylchloride or  
3 polyolefin film containing corresponding species of immobilized  
4 capture antibodies. The layer of immobilized antibodies is  
5 attached to the outer polymer film in predetermined patterns of  
6 simple icons, as best seen in Figures 7,7A. When the  
7 particular species of bound antigen encounters a particular  
8 corresponding species of immobilized antibody specific to a  
9 separate and distinct epitope thereof, further binding occurs.  
10 Upon the antigen binding to the two antibodies, a distinct icon  
11 shape emerges on the outer film at the point of binding,  
12 thereby providing a visual indicator.

13 While it is theoretically possible to detect an unlimited  
14 number of pathogens present in a packaged product, then to  
15 present this information in a very clear and unmistakable  
16 manner to an untrained consumer, as a practical matter there  
17 are limits to the amount of information which can be developed  
18 and presented in the biological material detecting system.  
19 Some of the limiting factors are cost, available surface area  
20 for display of information, complexity, and other  
21 considerations. Thus, for illustrative purposes only, the  
22 biological material detecting system as exemplified herein  
23 utilizes four separate pairs of antibodies, as set forth in  
24 Figures 7 and 7A. This is in no way meant to suggest a limit

1 on the number of antibodies that can be utilized in a single  
2 biological material detecting system.

3 As demonstrated in Figures 7 and 7A, the invention is  
4 exemplified with reference to detection of the following four  
5 microbes:

- 6 1. E-Coli;
- 7 2. Salmonella;
- 8 3. Listeria; and
- 9 4. Cyclospora.

10 To each of the four microbes, a particular icon shape is  
11 assigned. Although there are infinite numbers of icons which  
12 might be used including letters, numbers, or even words, we  
13 have chosen simple identifiers for the purpose of  
14 demonstration. As an initial step in the construction of the  
15 biological material detecting system, the outer polymer film or  
16 base layer undergoes a printing process in which a pattern of  
17 the four icons, wherein each icon utilizes a specific species  
18 of immobilized capture antibody, is applied thereto.  
19 Corresponding species of free antibodies, known as detector  
20 antibodies, which are biologically active ligands characterized  
21 by their ability to recognize a different epitope of the same  
22 particular toxic substance being tested for, and suspended in  
23 an agarose gel solution containing a surfactant and a nutrient,  
24 are printed in registration with the immobilized antibodies so

1 as to be in overlying and juxtaposed relationship thereto, and  
2 are then dried. Lastly, a second gel coat having a degree of  
3 porosity sufficient to prevent passage of the detector  
4 antibodies is laminated to the preparation.

5 Although the detection of biological materials through the  
6 use of antibodies is well known, there are several new and  
7 novel aspects to the application of antibody science which are  
8 set forth in the development of the biological material  
9 detecting system of the present invention.

10 Among these are: 1) the use of multiple antibodies to  
11 detect multiple biological materials in individual packages; 2)  
12 the use of a distinctive icon or other shape to not only  
13 detect, but visually identify the biological materials to the  
14 consumer, vendor, regulator, etc.; 3) insuring that detection  
15 and identification of the biological materials is accomplished  
16 in a timely manner in each particular application by  
17 judiciously controlling the porosity of the gel coat, thereby  
18 controlling the lapse rate of the reaction through the strength  
19 of capillary action; 4) inclusion of additives within the  
20 special gel coat to enhance the levels of microbes present; 5)  
21 incorporating the biological material detecting system of the  
22 instant invention within the existing packaging industry  
23 infrastructure; and 6) providing a bioassay material and  
24 methods for its production and use which immobilizes the

1 antibodies onto the surface of a flexible polyvinylchloride or  
2 polyolefin, e.g. a polyethylene, a surface treated  
3 polyethylene, a polypropylene, a surface treated polypropylene  
4 or mixture thereof.

5 The embodiment discussed above is based upon a sandwich  
6 immunoassay as depicted in Figure 1, which measures specific  
7 microbes, wherein the particular toxic substance is one or more  
8 members selected from the group consisting of a particular  
9 microorganism or species thereof, biological materials  
10 containing the genetic characteristics of said particular  
11 microorganism, and mutations thereof. In a particular  
12 embodiment, the toxic substance is selected from the group  
13 consisting of microorganisms, nucleic acids, proteins, integral  
14 components of microorganisms and combinations thereof.

15 It should also be understood that the invention will  
16 function by direct measurement of microbes with certain types  
17 of antibodies, selected from the group consisting of an  
18 antibody, a single stranded nucleic acid probe, an aptamer, a  
19 lipid, a natural receptor, a lectin, a carbohydrate and a  
20 protein. The biological materials may also be measured by non-  
21 immunological methods in particular using labeled molecules,  
22 such as aptamers, which have a high affinity for the biological  
23 materials.

24 The invention utilizes various types of detector

antibodies, e.g. those conjugated with dyes to produce a visual cue, or alternatively, photoactive compounds capable of producing a visual cue in response to a particular type of light exposure, for example a scanning system which detects luminescent properties which are visualized upon binding of the antigen and antibody. In this method of construction biological materials are measured directly with a biologically active ligand, e.g. an antibody, aptamer, nucleic acid probe or the like, which induces a conformational change to produce a visual cue.

It is also understood that specific polymers may be incorporated into the invention and that when a biological material is bound to the surface it induces a molecular change in the polymer resulting in a distinctly colored icon.

Referring to Figures 2 and 2A, in an alternative embodiment, a sandwich-type of construction is not necessary. As depicted in Figures 2 and 2A, the provision of certain types of biologically active ligand, e.g. chromogenic ligands to which receptors are bound will permit the visual confirmation of binding of the antigen to the immobilized ligand.

As depicted in Figure 3, a polymer film is provided and a biologically active ligand, preferably a chromogenic ligand, is immobilized to the polymer film. In the past, immobilized ligands were attached to rigid solid support matrices such as

1 plastic, polystyrene beads, microtitre plates, latex beads,  
2 fibers, metal and glass surfaces and the like. The immobilized  
3 ligands have also been attached to flexible surfaces such as  
4 nitrocellulose or polyester sheets which were not transparent.  
5 Surprisingly, the inventor has discovered that it is possible  
6 to attach biologically active ligands to the surface of various  
7 flexible polymeric films, for example polyvinylchloride and  
8 polyolefins, e.g. a polyolefin sheet having appropriate  
9 properties of transparency and flexibility and that the  
10 composite functions as a biological sensor or assay material.  
11 After printing on the polymer film, the material goes through  
12 a drying step; subsequent to which a special gel coat or liquid  
13 film is applied as a protectant layer and the final product is  
14 then dried.

15 Illustrative of films which will function in the present  
16 invention is a film containing a structural polymer base having  
17 a treated surface and incorporating therein a fluorescing  
18 antibody receptor and finally a stabilized gel coat. These  
19 films may be untreated polyethylene or polyvinylchloride films  
20 which are amenable to antibody immobilization by various  
21 mechanisms, e.g. by adsorption. In a particular embodiment,  
22 the films may be first cleaned, e.g. by ultrasonication in an  
23 appropriate solvent, and subsequently dried. For example, the  
24 polymer sheet may be exposed to a fifteen minute ultrasonic

1 treatment in a solvent such as methylene chloride, acetone,  
2 distilled water, or the like. In some cases, a series of  
3 solvent treatments are performed. Subsequently, the film is  
4 placed in a desiccating device and dried. Alternatively, these  
5 films may be created by first exposing the film to an electron  
6 discharge treatment at the surface thereof, then printing with  
7 a fluorescing antibody receptor. Subsequently, a drying or  
8 heating step may be utilized to treat the film to immobilize  
9 the receptor. Next, the film is washed to remove un-  
10 immobilized receptor; the film is then coated with a gel and  
11 finally dried.

12 Additional modifications to polyolefin films may be  
13 conducted to create the presence of functional groups, for  
14 example a polyethylene sheet may be halogenated by a free  
15 radical substitution mechanism, e.g. bromination,  
16 chlorosulfonation,, chlorophosphorylation or the like.  
17 Furthermore, a halodialkylammonium salt in a sulfuric acid  
18 solution may be useful as a halogenating agent when enhanced  
19 surface selectivity is desirable.

20 Grafting techniques are also contemplated wherein hydrogen  
21 abstraction by transient free radicals or free radical  
22 equivalents generated in the vapor or gas phase is conducted.  
23 Grafting by various alternative means such as irradiation,  
24 various means of surface modification, polyolefin oxidation,

acid etching, inclusion of chemical additive compounds to the polymer formulation which have the ability to modify the surface characteristics thereof, or equivalent techniques are all contemplated by this invention.

Additionally, the formation of oxygenated surface groups such as hydroxyl, carbonyl and carboxyl groups via a flame treatment surface modification technique is contemplated.

Further, functionalization without chain scission by carbene insertion chemistry is also contemplated as a means of polyolefin polymer modification.

Illustrative of the types of commercially available films which might be utilized are polyvinylchloride films and a straight polyethylene film with electron discharge treatment marketed under the trademark SCLAIR®. The electron discharge treatment, when utilized, renders the film much more susceptible to immobilization of the antibodies on its surface. Additional films which might be utilized are Nylon 66 films, for example DARTEK®, a coextrudable adhesive film such as BYNEL® and a blend of BYNEL® with polyethylene film.

With reference to Figures 4-6, one of the most important features of the biological material detecting system is its ability to quantitatively sensitize the antibody or aptamer so as to visually identify only those biological materials that have reached a concentration level deemed harmful to humans.



1 One means of providing this sensitization is by including a  
2 scavenger antibody which is a biologically active ligand  
3 characterized as having a higher affinity for the particular  
4 toxic substance than the capture antibody. The scavenger  
5 antibody is provided in a sufficient amount to bind with the  
6 particular toxic substance up to and including a specific  
7 threshold concentration. In this manner, the capture antibody  
8 will be prevented from binding with a detector antibody until  
9 the concentration of the particular biological material  
10 surpasses the specific threshold concentration. In this  
11 manner, the biological material detecting system visually  
12 reports only those instances where concentration levels are  
13 deemed harmful by health regulatory bodies.

14 Since the biological material detecting system as  
15 described herein can maintain its activity over long periods of  
16 time, e.g. up to 1 year, it is able to protect against  
17 contamination in products which have long shelf lives.  
18 Additionally, by reporting only toxic concentrations, it avoids  
19 "false positives" and, in some cases, can extend the useful  
20 life of the product.

21 Referring to Figures 9 and 10, the apparatus for producing  
22 the biological material detecting system is illustrated. These  
23 embodiments are essentially particular combinations of  
24 printers, coaters and dryers which will be used to place

1 biologically active reagents upon a thin polymer film useful  
2 for packaging food stuffs and other products. These films will  
3 be further processed subsequent to application of the  
4 biological material detecting system by printing, laminating,  
5 or equivalent methods of fabrication. The machinery is  
6 designed so that it will transport and process very thin films  
7 at rather high speeds. Furthermore, the machinery is designed  
8 so that it can be utilized effectively as an additional  
9 processing step when added to continuous processing operations  
10 already in use at packaging material fabrication plants. The  
11 printing machinery is designed so that a minimum of four  
12 distinct biological active ligands in a hydrate solution can be  
13 printed in patterns in a precise registration on the polymer  
14 film. The printing may be accomplished by jet spray or roller  
15 application, or equivalent printing methods. Each print  
16 applicator is capable of printing a detailed icon no larger  
17 than 1/4" x 1/4" in a minimum thickness. Patterning may be  
18 controlled by computer or roller calendaring. It is important  
19 to determine the appropriate viscosity of the solution to be  
20 applied so that successful printing, coating, and drying can be  
21 accomplished. After the printing step, the icons must be  
22 protected. This is accomplished by a final application of a  
23 thin special gel coat or a thin liquid film. By way of  
24 example, the liquid film may be an overprint food varnish.

1 This step is accomplished by a 100% coating of the entire film  
2 or alternatively by selectively coating each icon such that a  
3 10% overlap is coated beyond the icon in all directions. This  
4 coating step may be accomplished with sprays or rollers and the  
5 viscosity of the coating material must be optimized so as to  
6 provide adequate coverage. The biological material detecting  
7 system must be dried after printing and once again after  
8 coating. The drying is accomplished in a very rapid manner so  
9 as to enable high through put for the process. Various means  
10 of drying include the use of radiant heat, convected air and  
11 freeze drying. Care must be taken to avoid drying temperatures  
12 which will inactivate the biological reagents which have been  
13 applied. The polymer film which has been surface treated in  
14 the form of electron discharge, e.g. corona treatment, is most  
15 preferred. After preparation, the thin film is transported at  
16 relatively high speeds so that a wrinkle free surface is  
17 provided for printing, coating and rollup. Additionally, the  
18 apparatus provides a complete recovery system for the reagents  
19 which allows for total recovery of the agents and the volatile  
20 organic contaminants.

21 The invention will be further illustrated by way of the  
22 following examples:

#### 23 **EXAMPLE 1**

#### 24 **Detection of Antibody on the Surface of a Thin Layer**

**Polyvinylchloride Sheet:**

Rabbit polyclonal IgG was diluted to a final concentration of 2.0 µg/ml in 0.1M carbonate ( $\text{Na}_2\text{CO}_3$ )-bicarbonate ( $\text{NaHCO}_3$ ) buffer, pH 9.6.

Using a 2" x 3" grid, 75 µL (150 ng) was applied to a sheet of polyvinylchloride at 1" intervals.

The antibody treated polyvinylchloride sheet was dried for 1.5 hours at a temperature of 37°C.

The dried sheet was then washed 3 times with a phosphate buffered saline solution at a pH of 7.4.

HRP conjugated goat anti-rabbit IgG ( $\text{G}\alpha\text{R}^{\text{HRP}}$ ) was diluted to a concentration of 1:7000 in 1% casein, 0.1M potassium ferricyanide  $\text{K}_3\text{Fe}(\text{CN})_6$ , 0.1% phosphate glass ( $\text{Na}_{15}\text{P}_{13}\text{O}_{40}$  -  $\text{Na}_{20}\text{P}_{18}\text{O}_{55}$ ), at a pH of 7.4.

A precision pipette was used to apply 125 µL of diluted  $\text{G}^{\text{HRP}}$  to the grid backed polyvinylchloride sheet at 1" intervals coinciding with the area covered by the previously coupled  $\text{R}\alpha\text{G}$ . The sheet was incubated at room temperature for 30 minutes.

The sheet was then washed 3 times with phosphate buffered saline at a pH of 7.4.

To the test areas, 125µL of precipitating TMB enzyme substrate was added.

The sheet was incubated at room temperature until color development was complete.

Lastly, the sheet was washed 3 times with deionized water and allowed to air dry.

## EXAMPLE 2

Full Sandwich Immunoassay on the Surface of a Thin Layer  
Polyvinylchloride Sheet:

Rabbit polyclonal IgG was diluted to a final concentration of 2.0 µg/ml in 0.1M carbonate (Na<sub>2</sub>CO<sub>3</sub>)-bicarbonate (NaHCO<sub>3</sub>) buffer, pH 9.6.

A 13 x 9 cm piece of thin layered polyvinylchloride sheet was inserted into a BIO-RAD DOT-SPOT apparatus possessing 96 sample wells spaced at 1.0 cm intervals in a 12 x 8 well grid.

A 100  $\mu$ L sample (1.0  $\mu$ g) of rabbit polyclonal IgG was applied to each well of column 1 (8 wells total).

Antibody samples applied to columns 2-12 represented serial dilutions of the antibody ranging from 500 ng - 0.5 ng.

The antibody treated polyvinylchloride sheet was dried overnight at 37° C.

The dried sheet was washed 3 times with phosphate buffered saline (PBS), pH 7.4.

The antigen was diluted to a final concentration of 1.0 µg/ml in TRIS buffered saline (TBS) with 1% casein, pH 7.4.

Applied to each well of the apparatus was 100  $\mu$ L, representing 100 ng, of antigen, which was subsequently incubated at room temperature for 1 hour.

1 The polyvinylchloride sheet was washed 3 times with  
2 phosphate buffered saline (PBS), pH 7.4.

3 Detector mouse monoclonal antibody was diluted 1:625 with  
4 TBS containing 1% casein, 0.1M potassium ferricyanide  $K_3Fe(Cn)_6$ ,  
5 and 0.1% phosphate glass ( $Na_{15}P_{13}O_{40} - Na_{20}P_{18}O_{55}$ ), pH 7.4.

6 To each well of row 1, 100  $\mu$ L of the 1:625 dilution of  
7 detector antibody solution was applied.

8 Detector samples of 100  $\mu$ L applied to rows 2-7 represented  
9 serial dilutions of the antibody ranging from 1:1,250 to  
10 1:80,000. Dilutions of detector antibody were incubated on the  
11 polyvinylchloride sheet for 1 hour at room temperature.

12 The polyvinylchloride sheet was washed 3 times with  
13 phosphate buffered saline (PBS), pH 7.4.

14 To each well of the DOT-SPOT apparatus, 100  $\mu$ L of goat  
15 anti-mouse IgG<sup>HRP</sup> was added and allowed to incubate for one hour  
16 at room temperature.

17 The polyvinylchloride sheet was washed 3 times with  
18 phosphate buffered saline (PBS), pH 7.4.

19 To the test areas 100  $\mu$ L of precipitating TMB enzyme  
20 substrate was added.

21 The sheet was incubated at room temperature until color  
22 development was complete (see Figure 8).

23 Lastly, the sheet was washed 3 times with deionized water  
24 and allowed to air dry.

### EXAMPLE 3

#### Half Sandwich Competitive Immunoassay on the Surface of a Thin Layer Polyvinylchloride Sheet:

In this example, the printing of an icon shape is employed, such as an "X" 132, illustrated in Figure 13A. The icon is printed overlying a flexible film base layer 110, having two surfaces, prepared as previously described. A first layer 112 in an icon shape containing an overprint food varnish in combination with a capture antibody, referred to as a biologically active ligand, is printed over one surface of the base layer 110 to allow the immobilization of the biologically active ligand 120 to the surface of the film 110. A second layer 114, in register with the first 112, containing a buffer medium comprising a dye conjugated to a peptide 122, wherein the biologically active ligand has a degree of affinity for the peptide, is printed over the first layer 112 to allow contact for the peptide/dye conjugate 122 to bind to the immobilized ligand 120 (Figure 11) forming a homogeneous conjugate blend 124. At this point, the area printed on the film appears colored. The dye chosen for this example is a food dye giving a blue coloration, and therefore a blue "X" 132 is visualized (Figure 13A).

In a further example, an overprint food varnish in combination with a biologically active ligand is placed in

1 contact with a dye conjugated to a peptide to form a  
2 homogeneous conjugate blend 124 as the peptide binds to the  
3 biologically active ligand. A single layer 116 in an icon  
4 shape of the homogeneous conjugate blend may be printed onto a  
5 flexible film 110. Thus, as in Figure 12, a second layer  
6 introducing the peptide/dye conjugate is not required.

7 The antigen, a particular toxic substance 128, being  
8 sought is competitive with the peptide conjugating the food dye  
9 for binding to the immobilized biologically active ligand. As  
10 illustrated in Figures 15A, 15B, and 15C, when the antigen 128  
11 comes in contact with the biologically active ligand (Figure  
12 15A) having a degree of affinity for a particular toxic  
13 substance greater than that for the peptide, the antigen  
14 displaces the peptide, releasing the peptide/food dye conjugate  
15 122 (Figure 15B), thereby exhibiting loss of color within the  
16 icon "X" 134 (Figure 13B). As the peptide/dye conjugate is  
17 displaced, the particular toxic substance binds to the  
18 immobilized ligand 150 (Figure 15C) producing a visual signal,  
19 in this case loss of coloration within the "X", which is  
20 indicative of both the presence and identity of the particular  
21 toxic substance. So, when placing the flexible film in an  
22 environment which may contain a particular toxic substance,  
23 loss of coloration exhibited gives the user a visual cue to  
24 determine if the particular toxic substance is present. If no



1 loss of color is exhibited, the particular toxic substance is  
2 absent. The user may also monitor the film for a period of  
3 time sufficient to observe a visual signal in order to  
4 determine the length of time necessary before the particular  
5 toxic substance reaches a level deemed unfit for consumption.

6 Alternatively, the parts needed to make a second icon  
7 shape, such as a circle 140 surrounding the "X" 132 to a  
8 sufficient degree as to render the first icon shape invisible,  
9 comprising the peptide conjugated food dye in a layer, may be  
10 printed directly onto a flexible polymer film 110. Referring  
11 to Figure 14A, when surrounding the icon "X" 132 chosen with  
12 the same dye/peptide conjugate, the "X" is not visualized.  
13 Thus, when the antigen displaces the peptide and loss of color  
14 within the area of the "X" 134 occurs, the image will appear as  
15 a blue circle 140 with a white or colorless "X" 134 through or  
16 within it (Figure 14B).

17 Any type of dye may be chosen that is approved for use  
18 with food products. In addition, more than one biologically  
19 active ligand may be utilized and more than one peptide may be  
20 utilized. Once the peptide/dye conjugate is allowed to bind to  
21 the immobilized ligand, the area may be washed to remove any  
22 excess conjugate not bound by the ligand. A liquid film may  
23 also be applied as a protectant layer covering the homogeneous  
24 conjugate blend. Also, when incorporating a second icon shape,

1 a second dye may be utilized within the first icon shape, while  
2 still using a first dye, in order to create a visual signal in  
3 absence of a particular toxic substance and, in the presence of  
4 a particular toxic substance, create a visual color change  
5 within the first icon shape.

6 All patents and publications mentioned in this  
7 specification are indicative of the levels of those skilled in  
8 the art to which the invention pertains. All patents and  
9 publications are herein incorporated by reference to the same  
10 extent as if each individual publication was specifically and  
11 individually indicated to be incorporated by reference.

12 It is to be understood that while a certain form of the  
13 invention is illustrated, it is not to be limited to the  
14 specific form or arrangement herein described and shown. It  
15 will be apparent to those skilled in the art that various  
16 changes may be made without departing from the scope of the  
17 invention and the invention is not to be considered limited to  
18 what is shown and described in the specification and  
19 drawings/figures.

20 One skilled in the art will readily appreciate that the  
21 present invention is well adapted to carry out the objectives  
22 and obtain the ends and advantages mentioned, as well as those  
23 inherent therein. The embodiments, methods, procedures and  
24 techniques described herein are presently representative of the

preferred embodiments, are intended to be exemplary and are not intended as limitations on the scope. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention and are defined by the scope of the appended claims. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art are intended to be within the scope of the following claims.